

# The Effects of Human New Pressor Protein and Coagulation Factor XIIa on Blood Pressure and Heart Rate in Bilaterally Nephrectomized Rats

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## Abstract

**Background:** New Pressor Protein (NPP) is a human plasma enzyme structurally related to  $\beta$ -fragment of activated factor XII ( $\beta$ -FXIIa). The objective of the present study was to investigate the effects of NPP and  $\beta$ -FXIIa on systolic blood pressure and heart rate in bilateral nephrectomized rats.

**Methods:** Forty male Wistar rats (250-300 g) were sham-operated or bilaterally nephrectomized under anesthesia with a combination of halothane, nitrous oxide and oxygen. Twenty four hours later under anesthesia with Inactin (100 mg/kg), they were ganglion blocked (pentolinium tartrate; 19.2 mg/kg), and their systolic blood pressure and heart rate were measured before and after intravenous administration of captopril (2.5 mg/kg), NPP (20  $\mu$ l plasma equivalent) or purified  $\beta$ -FXIIa (300 ng/kg).

**Results:** NPP raised the systolic blood pressure by  $31 \pm 2$  mmHg and heart rate by  $19 \pm 2$  bpm in sham-operated rats. Captopril caused systolic blood pressure and heart rate to increase significantly by  $64 \pm 7$  mmHg and  $107 \pm 9$  bpm, respectively in response to NPP. In bilateral nephrectomized rats, NPP raised systolic blood pressure by  $57 \pm 6$  mmHg and heart rate by  $70 \pm 13$  bpm in the absence of captopril, which were not significantly different from those (46  $\pm$  3 mmHg and 75  $\pm$  8 beats/min) in the presence of captopril.

**Conclusion:** This study shows that the effects of NPP and  $\beta$ -fragment of factor XII on systolic blood pressure and heart rate are similar, suggestive of a functional relationship between them. The findings might suggest that the potentiation effect of captopril in sham-operated or bilaterally nephrectomized rats is primarily expressed via renal enzymes.

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**Keywords** • Nephrectomy • captopril • factor XIIa

## Introduction

New Pressor Protein (NPP) is an extrarenal enzyme derived from trypsin-activated normal human and rat plasmas. Biochemical studies of human NPP revealed that it was a heat labile enzymatic protein with a molecular mass of ~30 KDa and an isoelectric point of 4.7 to 4.9. Its amino-terminal sequences (19 amino acid residues) indicated strong homology

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with the heavy chain of the  $\beta$ -fragment of activated human coagulation factor XII ( $\beta$ -FXIIa).<sup>1,2</sup> Structural and functional properties of NPP indicate that its effects can not be attributed to any identifiable pressor agent including angiotensin II or endothelin.

Intravenous physiological bolus doses (10-20  $\mu$ l plasma equivalent /~300g) of impure human or rat NPP in rats produced biphasic or multiphasic blood pressure responses. They were associated with a small and brief initial depressor phase followed by a much more prominent and prolonged (10-15 min) pressor phase accompanied by a marked elevation in heart rate (HR).<sup>3</sup>

In a series of preliminary experiment, we found that bilateral nephrectomy (2NX) potentiated NPP responses. Hypertension is a common problem among pediatric hemodialysis patients.<sup>4</sup> The present study aimed at determining further the mechanism of action of NPP and the role of kidneys on its responses.

## Methods

### Statement of ethical guidelines

Animal used in the present study were cared for and used in accordance with the principles and guidelines outlined by the Canadian Council on Animal Care. All experimental protocols were approved by the relevant Animal Care Committees of the Faculty of Medicine and the University of Toronto.

### Surgery and instrumentation

Male Wistar rats (n=40) weighing 250-300 g were obtained from Canadian Biobreeding Laboratories. Animals were subjected to sham-operation or nephrectomy as described previously.<sup>5</sup> Briefly, under anesthesia by a mixture of halothane and nitrous oxide in pure oxygen the hairs of animals backed at the level of last rib and the flank areas were shaved, and the shaved areas were antisepticised with a 70% ethanol. A mid-line skin incision was made above the spinal cord. Through the incision, the skin around it was separated laterally by blunt dissection. Afterwards, the incision was pulled to the right and left. An incision was made in the muscular layers of the flank areas, and the kidneys were exposed. The adrenal vessels were tied with a 1-0 silk thread temporarily, and the kidneys were decapsulated to preserve the adrenal gland and removed. The sham-operated rats were subjected to a similar operation, but kidneys were not removed. The muscular incision was sutured with a 3-0 silk thread, and the mid-line skin incision was closed with a 9 mm stainless steel autoclip. The animals were allowed to recover from

anesthesia, and kept in cages with free access to water and rodent chow.

Twenty four hours later, animals were anesthetized with Inactin (100mg/kg), and right carotid arteries were cannulated using PE-50 polyethylene catheters for arterial systolic blood pressure (SBP) and HR recordings, using Statham DC pressure transducers (Hato Rey). The transducers were connected to a MacLab/8 data acquisition system (AD Instruments and Lamont Scientific, Toronto, Canada) connected to a Power Macintosh 7200/1200 PC compatible computer and driven by MacLab Chart Version 3.5.6 software. Only rats that had sustained minimal tissue trauma and blood loss, and had stable BP (~80/40 mmHg, systolic/diastolic blood pressure) and HR (~350 beats/min) were used for the experiments, otherwise excluded. All the animals received single subcutaneous injections of ganglion-blocking agent pentolinium tartrate (19.2 mg/kg) and atropine sulphate (2.4 mg/kg) during surgery.

### Experiments design and protocol

The rats were assigned to two series. The first series were 2NX rats including two groups (n=8-10 each) receiving captopril or vehicle. The second series were sham-operated rats divided into 2 groups (n=8-10 each) in a similar manner as 2NX rats. Captopril (2.5 mg/kg) was administered intravenously 40 min before the SBP and HR recordings. Animals in each group received single intravenous injections of NPP (20  $\mu$ l plasma equivalent) and  $\beta$ -FXIIa (300 ng/kg), SBP, or HR increment was calculated at the peak of blood pressure responses.

### Human plasma and preparation of NPP

Human plasmas that were considered normal but unsuitable for transfusion purposes, were obtained from the Canadian Services (formerly the Canadian Red Cross Society Toronto Center, ON, Canada). The plasma bags were thawed in cold tap water, and aliquots were used immediately or frozen at -20°C in capped polystyrene tubes for later use.<sup>1</sup>

Plasma was activated with trypsin in a controlled reaction, as described previously.<sup>1,3</sup> Briefly, trypsin (type III, bovine, T-8253; Sigma Chemical Co., St Louis, Missouri, USA) was prepared as a stock solution in 0.002 mol/l HCl and added to plasma, with mixing at 3-10% v/v to achieve a final trypsin concentration of 1 mg/ml ensuring minimal plasma dilution. After incubation for 10 min at 23°C, the reaction was terminated by rapid freezing on dry ice. The activated plasma preparation was administered to rats at a dose of 20  $\mu$ l/300g rat, expressed in terms of plasma equivalence. The pressor

properties of this preparation were shown to be virtually interchangeable with our own highly purified preparations of NPP<sup>3</sup> or with a commercially obtained highly purified  $\beta$ -FXIIa fragment.<sup>1</sup>

#### Drugs

The ganglion-blocking agent pentolinium tartrate salt (P-3520, Sigma-Aldrich, Poulenc, Montreal, Canada) was dissolved in poly vinyl pyrrolidine for continuous release during the experiment. Inactin (Promonta, Hamburg, Germany) and captopril (Sigma-Aldrich C-4042, St Louis, Mo, USA) were dissolved in 0.9% saline. Atropine sulphate was obtained from Ingram and Bell (Toronto, ON, Canada). Human coagulation  $\beta$ -FXIIa was obtained from Enzyme Research Laboratories (South Bend, IN, US). Aliquots of  $\beta$ -FXIIa were prepared in concentrations of 10 ng/ $\mu$ l, and were stored at -40°C. The aliquots were kept on ice during the experiments.

#### Statistical analysis

Results are expressed as Mean  $\pm$  SEM. Statistical comparisons were made using either one-way analysis of variance, followed by Duncan's Multiple Range test, or paired or unpaired Student *t* test where appropriate. A  $P \leq 0.05$  was considered statistically significant.

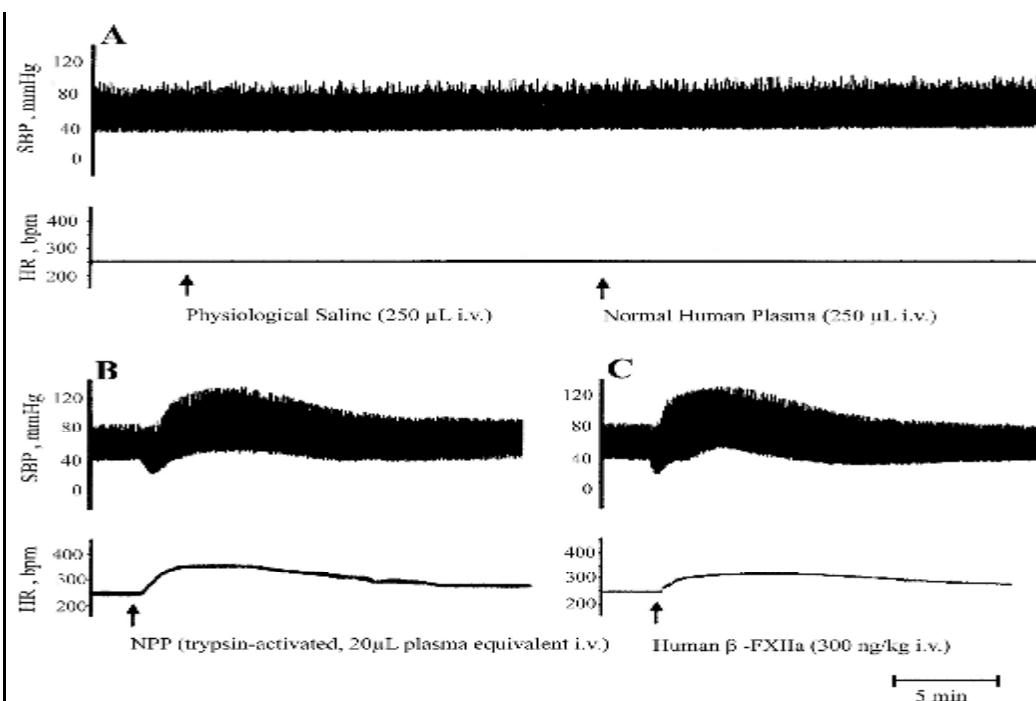
## Results

#### The effects of intravenous injections of human NPP and purified human $\beta$ -FXIIa in sham-operated rats

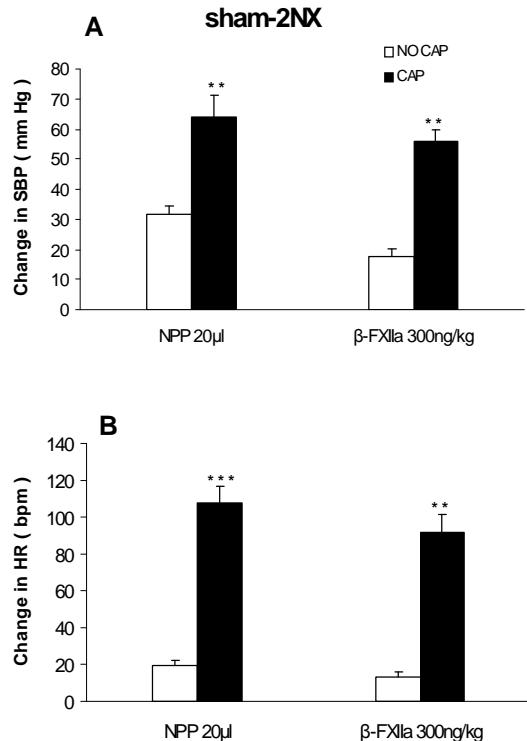
The injections of physiological saline or normal human inactivated plasma did not change the SBP and HR in rats pretreated with captopril (fig 1-A). However, NPP and purified human coagulation  $\beta$ -FXIIa produced a biphasic effects on SBP comprising of an initial small decrease followed by a much larger increase accompanied by an increase in HR lasting for approximately 15 min (fig 1 B, C).

#### The effect of captopril pretreatment on SBP and HR responses to NPP and $\beta$ -FXIIa in sham-operated rats

The administration of NPP and  $\beta$ -FXIIa to sham-operated rats caused increases in SBP of 31.7  $\pm$  2.0 and 17.6  $\pm$  2.0 mmHg, respectively. They also caused increases in HR 19.8  $\pm$  2.0 and 13.6  $\pm$  2.0 bpm, respectively. The administration of NPP and  $\beta$ -FXIIa after pretreatment with captopril increased SBP 64.2  $\pm$  7.0 and 56  $\pm$  4.0 mmHg, respectively. Also they caused increases in HR 108  $\pm$  9 and 92  $\pm$  10 bpm, respectively. The increases in SBP and HR caused by both compounds in the presence of captopril were significantly higher than the respective values in the absence of pretreatment with captopril (fig 2).



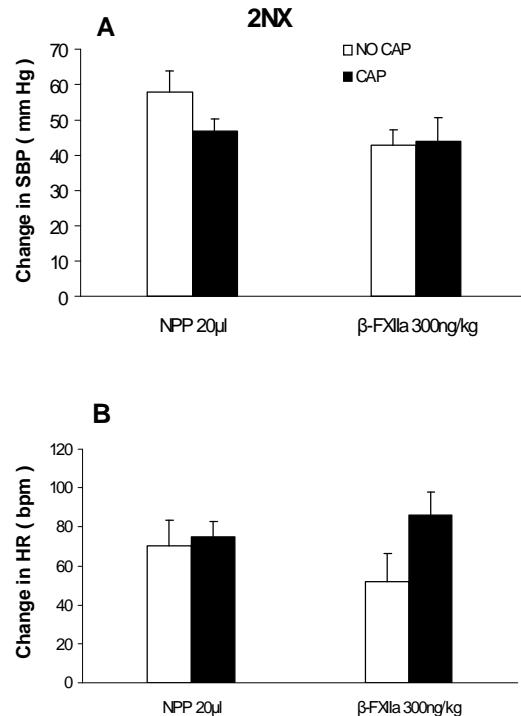
**Fig 1:** Representative systolic blood pressure (SBP, mmHg) and heart rate (HR, beats per minute, bpm) responses to physiological saline, normal inactivated human plasma (A), to human NPP (20  $\mu$ L plasma activated equivalent i.v.) (B) and coagulation  $\beta$ -FXIIa (300 ng/kg) (C). Human NPP and highly purified  $\beta$ -FXIIa produced comparable SBP and HR responses in bioassay rats lasting ~15 min. Physiological saline or normal (control) human plasma preparation not activated with trypsin produced no change in these responses.



**Fig 2:** Effect of human NPP (20 $\mu$ l plasma activated equivalent, i.v.) or  $\beta$ -FXIIa (300 ng/kg, i.v.) on systolic blood pressure (SBP, mmHg) (A) and on heart rate (HR, bpm) (B) in sham-operated rats. Open bars show responses before captopril and dark bars show responses after captopril (CAP, 2.5 mg/kg, i.v.) injection. There are significant differences between these groups before and after captopril treatment. \*\*P<0.01, \*\*\*P<0.001. (Mean  $\pm$  SEM, n=8-10).

#### The effect of captopril pretreatment on SBP and HR responses to NPP and $\beta$ -FXIIa in 2NX rats

The administration of NPP or  $\beta$ -FXIIa to nephrectomized rats evoked marked increases in SBP  $57.7 \pm 6.0$  and  $42.9 \pm 4.0$  mmHg, respectively. They also caused increases in HR  $70.4 \pm 13$  and  $51.7 \pm 14$  bpm, respectively. The responses started with a brief decrease, followed by a quick increase in SBP and DBP. After the quick rise, pressure began to fall and reached to baseline within 15 minutes. The administration of NPP and  $\beta$ -FXIIa after pretreatment with captopril caused increases in SBP  $46.7 \pm 3.0$  and  $43.9 \pm 6.0$  mmHg and increases in HR  $75.1 \pm 8.0$  and  $85.0 \pm 12.0$  bpm, respectively (fig 3). These values were not significantly different from identical values in the absence of captopril.



**Fig 3:** Effect of human NPP (20 $\mu$ l plasma activated equivalent, i.v.) or  $\beta$ -FXIIa (300 ng/kg, i.v.) on systolic blood pressure (SBP, mmHg) (A) and on heart rate (HR,bpm) (B) in 24 hr nephrectomized (2NX) rats. Open bars show responses before captopril and dark bars show responses after captopril (CAP, 2.5 mg/kg, i.v.) injection. There are no significant differences between these groups before and after captopril treatment. (Mean  $\pm$  SEM, n=8-10).

#### Discussion

The findings of the present study indicate that the administration of captopril in sham-operated rats as well as nephrectomy potentiated the increase in SBP and HR evoked by NPP and  $\beta$ -FXIIa. It also showed that captopril failed to potentiate such responses in 2NX rats (fig 3).

Trypsin-activated normal human plasma produced a characteristic pressor SBP and HR responses (fig 1-B). In contrast, untrypsinized human plasma or saline solution exhibited no responses (fig 1-A). NPP is homologous to  $\beta$ -FXIIa, which has similar pressor and chronotropic characteristic (fig1-C). Structural and enzymic studies confirm the relationship between NPP and  $\beta$ -FXIIa.<sup>1-3</sup>

Since trypsin can activate pro-renin to renin, which produces pressor effects in 2NX rats,<sup>3</sup> it is expected that renin angiotensin system

might be involved. However, the present study showed that pretreatment with captopril in ganglion-blocked sham-operated rats not only could prevent pressor or HR responses but also potentiated such effects (fig 2-A, B). Similar effects were also reported with enalapril suggesting that ACE inhibition could potentiate NPP effects.<sup>1,3</sup> Moreover, it was reported that peptides such as bradykinin and pituitary adenylate cyclase activating polypeptide (PACAP) might mediate NPP responses.<sup>6,7</sup> Therefore, it is likely that captopril did potentiate the responses to NPP and  $\beta$ -FXIIa via the preservation of these peptides (fig 2-A, B). Since captopril pretreatment could not lower BP response to NPP, renin angiotensin system might not be involved in NPP pressor effects.

Hypertension is prevalent among pediatric hemodialysis patients.<sup>4</sup> The role of NPP in hypertensive hemodialysis patients is not known. In a previous study we showed that plasma levels of NPP and  $\beta$ -FXIIa were high in hemodialysis patients.<sup>8</sup> The present study used 24 hrs 2NX rats to investigate the role of NPP and the role of ACE inhibition in NPP effects. The 2NX rats were chosen, since the removal of renal ACE was expected to simulate the potentiating effects of captopril. The findings suggest that the effects of NPP and  $\beta$ -FXIIa on SBP, HR, and the potentiation of their effects by captopril were quite similar in sham-operated rats (fig 2-A, B). However, 2NX alone produced virtually the same potentiation of responses to NPP and  $\beta$ -FXIIa as did captopril in sham-operated rats (fig 3-A, B). This finding might suggest that the effects of captopril were primarily expressed via renal peptidases. Since tissues other than kidney have ACE, captopril was not able to increase more the effects of NPP and  $\beta$ -FXIIa in 2NX rats. This suggests that the potentiating effect of 2NX on SBP and HR responses might be due to the removal of other renal peptidases such as neutral endopeptidase (NEP).<sup>9</sup> Thus, it is a non-clearance mechanism and is due to the preservation of peptides, which mediate catecholamine release. These peptides are sensitive to biodegrading by ACE and NEP.<sup>10</sup>

Another possibility for the potentiating effect of 2NX might be the elevation of extrarenal pro-renin and angiotensinogen levels.<sup>11,12</sup> NPP or  $\beta$ -FXIIa might produce a significant amount of angiotensin II by converting pro-renin to renin in such a model. Angiotensin II increases BP and thus potentiates NPP responses. However, pretreatment of 2NX rats with Losartan, an AT-1 receptor antagonist, did not change NPP pressor effects,<sup>7</sup> therefore, the role of RAS in NPP response remains equivocal.

The fact that captopril potentiated HR responses more than SBP ones to NPP in sham-operated rats (444% vs 102%) suggests that the mechanisms governing HR are not entirely similar to those governing SBP. Overall, the findings of the present study and those from previous ones,<sup>1-3,13</sup> solidify the relationship between NPP and  $\beta$ -FXIIa, and demonstrate that complex mechanisms of action on SBP and HR are importantly mediated by the kidneys.

In conclusion, the findings strongly support the similarity between NPP and  $\beta$ -FXIIa effects for elevating SBP and HR. These responses are potentiated in nephrectomized rats, suggesting the involvement of kidneys. In patients with renal ablation or renal failure, this hypertensive effect might exist.

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